HEMOLYTIC STREPTOCOCCI FOUND IN MILK

THEIR SIGNIFICANCE AND THEIR RELATION TO VIRULENT STREPTOCOCCI OF HUMAN ORIGIN*

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The relation of epidemics of streptococcal sore throat to the milk supply has directed the attention of recent workers to the pathogenic properties of the various streptococci found in milk. So far as is known all streptococci responsible for the epidemics have been of the hemolytic type, and altho an enormous amount of work has been done on milk streptococci, comparatively little has been done on the hemolytic varieties in milk, particularly in relation to pathogenesis.

By the term hemolytic streptococci is meant those streptococci which on plain-blood-agar plates cause a zone of hemolysis about the colony. This zone is usually quite clear, and varies from 1 to 3 mm. in diameter. The organisms causing atypical greenish brownish zones, or those which are very feebly hemolytic, as Streptococcus viridans or the pneumococcus at times may be, especially when the blood is very dilute, are not included in this group. Furthermore, it is to be understood that the hemolytic streptococci do not constitute a single variety, or genus. Hemolysis is a property common to a number of kinds of streptococci that might differ from one another decidedly in other respects. It is variable, at least within certain limits, but nevertheless sufficiently stable to be a very useful property for many practical purposes.

Ruediger[1] in 1912 showed that hemolytic streptococci occur in milk. In his article he assumes that hemolytic streptococci and Streptococcus pyogenes are identical. He concludes from his study that Streptococcus pyogenes seems to occur but rarely in milk and that when it does, it is indicative of the existence of an inflamed condition of the udder of the cow. Streptococcus lacticus, he asserts, differs from Streptococcus pyogenes in that the colonies of the former are greenish, with little or no hemolysis, whereas those of the latter are surrounded by a large zone of hemolysis. He considers that Streptococcus lacticus has no sanitary significance whatever, while the hemolytic variety is to be looked on with suspicion. He isolated in a number of instances hemolytic strains from the diseased udders and teats of cows.

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[1] Science, 1912, 35, p. 223.

When Dr. Capps and I studied the milk epidemics of sore throat in Chicago in 1911 and in Jacksonville, Illinois, in 1914, we isolated from the milk direct from the diseased udder of certain cows strongly hemolytic streptococci in pure culture. They resembled the human strains isolated from sore throats. Strains of streptococci were isolated from milk from other diseased cows which were not hemolytic, and which differed in other respects from the human strains. Our results were published in a paper,[2] the introductory statement of which reads as follows:

"The Chicago epidemic of septic sore throat reported by Capps and Miller was traced to a contaminated milk supply at Batavia, Ill. It was found that on a number of farms supplying milk to Dairy X, sore throat occurred in the milkers and mastitis appeared in the cows; in some instances the sore throat appeared first and in other instances the mastitis preceded the throat infections. These facts bring up the question whether or not it is possible for milkers suffering from sore throat to infect the teats or udders of cows by contaminated hands or otherwise during the process of milking. The question of the susceptibility of the cow to human streptococci is also involved. In an attempt to clear up these points some experiments were designed to test the pathogenicity of human streptococci for cows and to determine possible avenues of udder infection through the teats."

It was clearly shown in our experiments that hemolytic streptococci of human origin, when introduced by catheter into the healthy udder of a cow, continue to grow and are shed for a considerable period of time, and that this is accompanied by certain evidences of mastitis. Furthermore, experiments made by swabbing human streptococci about the meatus of the healthy teat did not give rise to an ascending infection, but when the teat was injured by scarifying, the streptococci ascended the ducts and caused an infection manifested by marked increase in leukocytes, the presence of large numbers of the hemolytic streptococci, but not by any physical signs. The streptococci continued to be shed in the milk for a period of at least 4 weeks, at the end of which there were still large numbers of streptococci and leukocytes in the milk. This work has been confirmed and extended since by Dr. George Mathers (p. 222) in this number), who has studied especially the behavior and effects of streptococci of human and bovine origin when grown for a long period of time in the udder of the cow.

In a recent paper Theobald Smith and J. H. Brown[3] showed that streptococci which were the agents of a number of outbreaks of sore throat were "all alike in that the colonies produce immediately around them a clear hemolysed area on blood agar plates (horse blood)." They state: "Our studies extending over more than a year and a half have shown that cultures from throats affected with tonsillitis contained at least two types of streptococci well differentiated on horse blood agar plates. Our attention was largely restricted to one of these types, a streptococcus producing around the colony a clear zone three to four millimeters in diameter. This type corresponds with the hemolytic strains of earlier milk-borne epidemics of tonsillitis (Boston, Chicago, and Baltimore). Within each of these groups a close analysis of morphological characters did not bring out differences beyond slight variations in size of the cocci, but on culture media differences were evident." They divide streptococci on the basis of hemolysis into 2 types: Type A, in which the colony has a partly discolored and hemolyzed mantle between it and an outer clearer zone; and Type B, in

[2] Capps and Davis: Jour. Infect. Dis., 1914, 15, p. 135.
[3] Jour. Med. Research, 1914, 31, p. 455.

which the colony is surrounded by a clear zone of hemolysis. The former are nearly always nonpathogenic for rabbits; the latter are commonly pathogenic for rabbits, tho many strains are not. The cow streptococci were nonpathogenic for rabbits except 2 strains of the B type and possibly 1 of the A type. Smith and Brown, in looking for a sufficient reason for these explosive epidemics of tonsillitis in addition to the possible contamination of the milk during milking or later, point to the possibility of the occasional infection of the udder ducts with human streptococci. Such a possibility would be supported by the discovery of hemolytic streptococci of the human type in the milk (Outbreak B) from a cow in the suspected herd; also by the discovery in the mixed milk in the study of another epidemic (Outbreak G) of a strain not distinguishable from human pathogenic strains. The ordinary mastitis or garget streptococci, he infers, are different from streptococci of human tonsillitis and do not cause throat infections in man. Only rarely might human types find their way into the ducts through manipulations, and continue to be shed into the milk for some time.

Smith and Brown did not test this point experimentally on cows. It is evident, however, that the assumption made by these writers as regards the possible occurrence of streptococci of the human type in udders of cows was directly tested and supported by the experimental evidence obtained by Dr. Capps and myself previously, and this is pointed out by them in their paper.

Recently Krumwiede and Valentine[4] reported a milk epidemic in Rockville Centre, Long Island, in which they found in one cow the wide-zone hemolytic streptococci of the human type. They present evidence which they have interpreted as showing that the epidemic was largely caused by the udder infection of the cow, but that, in conformity with the assumption here discussed, this cow had itself been infected with human streptococci from a milker who was suffering from sore throat. The cow showed no evidence of mastitis except the flocculent character of the milk from one quarter. In identifying streptococci from human and bovine sources they insist on cultural identity in every detail, or immunologic identity. They give no data as to the virulence of their strains of streptococci for animals.

Savage[5] several years ago carried on certain experiments which have a distinct bearing on this problem, tho not altogether comparable with our own because made with goats and mice or guinea-pigs. The determination of the streptococci was also made on a different basis, no attention being given then to their reaction on blood media. However, his work points to the existence of 2 quite distinct types of streptococci as the cause of mastitis in cows, the one virulent for rodents and avirulent for goats, the other virulent for goats and avirulent for mice. The former agree in the main with the human type of streptococci which are found in infected throats, but which only rarely are found as the cause of mastitis in cows. The second type, however, is the common cause of mastitis and this organism Savage calls Streptococcus mastitidis. He tested Streptococcus mastitidis on man by inoculating his own throat with massive doses of this culture. In 2 separate experiments no ill effects were observed. It should be pointed out that he did not test on man the other variety, but he makes this statement: "In one case, for example, a streptococcus of extremely high virulence to rodents was found to be the cause of the mastitis and this organism in many other ways was quite distinctive from the streptococcus mastitidis and may well have been potentially virulent to man. If we accept the view that the ordinary type of bovine mastitis is due to organisms

[4] Jour. Med. Research, 1915, 33, p. 231.
[5] Milk and the Public Health, 1912.

non-virulent to man, but that in certain uncommon cases this condition is caused by streptococci highly pathogenic to man, it offers a complete explanation of both the bacteriological investigation and the epidemiological facts. From a practical point of view it is well to remember that the pathogenic and non-pathogenic types of bovine mastitis are not clinically distinguishable."

I wish to point out that, tho the data presented by Savage do not permit us to compare this organism, which he mentions as possibly pathogenic to man, with the human type in respect to hemolysis and some other characteristics, it appears most probable from the facts mentioned that he encountered this type in this instance. His data therefore are in entire accord with the data already analyzed in connection with this problem of the possible existence of a human and a bovine type of streptococcus as the cause of mastitis in cows.

Stokes and Hachtel[6] in their work on the Baltimore epidemic isolated a streptococcus of the epidemicus type from the mixed milk from one dairy, which conformed in all essential properties with the type from the human cases of septic sore throat. Other types of organisms and also slightly virulent pneumococci were isolated from milk which was suspicious on account of the high pus content.

In a number of other epidemics of milk origin, hemolytic streptococci virulent for animals have been obtained from milk from cows suffering with mastitis and some of these strains belong to the human type, as in the epidemics reported by North, Avery and White[7] and by Rosenow and Moon.[8]

While the hemolytic type of streptococcus has thus far been the causal agent in probably all epidemics, the possibility of nonhemolytic streptococci being pathogenic for man should be kept in mind and, as Theobald Smith says, the success in tracing an epidemic to its source depends on a detailed study of individual strains of streptococci and the discovery of certain minor distinguishing characteristics as guides. All hemolytic varieties are not necessarily pathogenic for animals or for man. Neither are all nonhemolytic streptococci avirulent. But these latter are not, so far as we know, concerned in causing epidemic septic sore throat.

In this connection it may be stated that nonhemolytic or feebly hemolytic streptococci may cause a distinct and long enduring mastitis in cows. I have isolated 3 such strains in pure culture directly from the inflamed udder of as many cows. They appear in the gargety milk in long chains and in large numbers. These organisms are harmless for rabbits unless one injects large doses (2 or more blood slants); then arthritis may develop. My impression from the data in the literature and also from my own experience is that such streptococci are not infrequently the cause of mastitis, but there is no evidence at present indicating that they are dangerous to man.

[6] Public Health Reports, 1912, 27, p. 1923.
[7] Jour. Infect. Dis., 1914, 14, p. 124.
[8] Ibid., 1915, 17, p. 69.

Organisms of this type include many of the streptococci in milk usually designated as Streptococcus lacticus. Most of these cause a green discoloration in blood media, but some may be feebly hemolytic, conforming in this regard with Smith and Brown's Type A; others cause no appreciable alteration of the surrounding media. These organisms likewise are relatively avirulent and, so far as is now known, possess no sanitary significance.

In view of the facts just stated it was thought desirable to study further the hemolysing streptococci from samples of milk obtained under various conditions, including both pasteurized and certified milk. From such milk a collection of strains was isolated and subjected to various tests, and was also compared in different ways with a collection of human hemolytic streptococci, particularly in regard to their pathogenicity for animals. Special attention was given to the study of the property of heat resistance on account of its relation to pasteurization. Only those streptococci were selected the colonies of which were surrounded by a distinct clear zone of hemolysis on human-blood-agar plates. (Type B, Theobald Smith.) The feebly hemolytic streptococci (Type A) were often noted in the milk, but were disregarded, since the interest in sore-throat epidemics has centered about the cocci with a clear wide zone. Other bacteria, both cocci and bacilli, were met which gave hemolytic colonies on blood plates similar to streptococci, but these were carefully excluded by suitable tests. Streptococcus mucosus was not encountered.

Three hundred twenty-eight specimens of bottled milk were collected from 9 different dairies in the city of Chicago. In Table 1 is given a summary of the results of the examinations made. All the specimens were pasteurized except the samples from Dairy 1, which furnished certified milk; and with the exception of the samples from Dairies D and E the holding process was used.

These examinations were made from October 1914 to March 1915. During the winter months the bacterial count on the whole was lower. The examinations were made shortly after the samples of milk had been delivered at the laboratory. Sometimes the bottles were kept in an ice box for a few hours.

Blood agar (human) was used in planting and the counts were made after incubation at 37 C. for 48 hours. The colonies of hemolytic streptococci were carefully noted and counted on the plates and later their identity was confirmed by further tests.

The lowest total bacterial counts occurred in the certified milk, the average of 45 samples being 12,306. Milk from certain of the daries ran consistently high in bacterial counts (G and H); that from others consistently low (B).

Eighty-five samples yielded on culture streptococci of the strongly hemolytic variety. From Table 1 it is seen that the number in different samples varies considerably, ranging from a few hundred to several thousand to the cubic centimeter. The certified and the pasteurized samples contained about equal

numbers. In 16 specimens of milk from Dairy B no hemolysing streptococci were found, but this was the only exception.

A study of the 85 strains of hemolysing streptococci was made as regards their morphology, cultural characteristics, and certain other properties.

The shape of these cocci varies; it is often spherical, but frequently is more or less elongated; some strains have the stockade appearance. Individual strains under different conditions and on the various media vary somewhat.

The arrangement in milk is usually in the form of a diplococcus; short chains of 3 or more are however very common. The arrangement depends largely on the medium. At times long chains may form, especially in broth. This feature is of little value in classifying the organisms.

TABLE 1

HEMOLYTIC STREPTOCOCCI IN MILK FROM VARIOUS DAIRIES

Dairy	Number of Samples	Range of Bacteria per c.c.	Average Number Bacteria per c.c.	Samples Containing Hemolytic Streptococci		Range of Hemolytic Streptococci per c.c.	Average Number Streptococci per c.c.
				Number	Percentage		
A	77	6,000 – 125,000	30,416	5	6.4	200 – 600	418
B	16	4,600 – 29,200	13,096	0	0.0	0	0
C	41	12,000 – 90,000	43,400	10	24.4	200 – 8,000	2,375
D	8	17,600 – 120,000	55,449	1	12.0	500	500
E	9	18,000 – 60,000	31,220	2	22.0	300 – 500	400
F	22	11,000 – 51,000	26,131	6	27.2	250 – 600	408
G	11	36,000 – 240,000	129,300	5	45.4	1,500 – 8,000	4,400
H	99	16,700 – 168,000	76,462	38	38.3	200 – 18,000	2,690
I*	45	6,900 – 240,000	12,306	18	40.0	200 – 3,000	1,028
Total	328	.....	.....	85			

* Certified.

They are in general distinctly gram-positive, tho a few strains at times stain irregularly by this method.

In plain broth their growth is variable. The turbidity is usually distinct. In many strains a fairly abundant sediment settles out with clearing of the medium, the sediment often adhering to the sides of the tube. They grow more profusely on the whole in this medium than do ordinary human strains of Streptococcus pyogenes. The addition of serum to broth increases the growth.

All the cultures when inoculated into litmus-milk tubes multiply at a temperature of 20 C. The milk is slowly acidified and later coagulated. In 24 hours many strains have turned the milk pink at this temperature; other strains grow

more slowly but at the end of 4 or 5 days all the tubes have turned. At 37 C. the milk is rapidly acidified and coagulated; in the lower part of the tube often the milk is pale pink or nearly white, while near the surface it is a deeper shade of red.

Of 79 strains of these streptococci tested on 8 sugars, all fermented dextrose, lactose and maltose, 11 failed to ferment saccharose, 65 failed to ferment mannite, 11 failed to ferment salicin, 3 fermented inulin, and 5 fermented raffinose.

Titration data of 14 milk strains on various carbohydrate media are given in Table 2. The figures represent the percentages of normal acidity developed in 1% sugar broth in 1 week at 37 C. The first 10 strains in the table are the milk strains described in the foregoing. The last 4 are virulent hemolytic streptococci, 3 of which were isolated from human lesions and 1 (No. 6) was isolated from the udder of a cow.

A study of hemolysis was made both on the original plates, and on plates subsequently made. The zone is usually clear, and the corpuscles immediately beneath and around the coloring are disintegrated. The diameter of the hemo-

TABLE 2

ACTION OF MILK STRAINS AND HUMAN STRAINS ON CARBOHYDRATES

Strains of Strepto-coccus	Glucose	Lactose	Maltose	Saccha-rose	Salicin	Mannite	Raffi-nose	Inulin
72	6.05	4.37	5.05	1.1	6.05	3.35	1.0	0.95
183	6.15	5.05	5.05	0.9	5.05	1.00	1.0	0.95
300	6.25	4.55	5.55	1.5	5.05	1.0	1.1	1.25
134	6.37	4.55	4.05	4.6	4.55	0.98	0.85	1.08
290	5.05	4.65	3.35	5.05	5.05	0.95	0.83	1.20
41	6.55	3.65	5.25	4.25	4.05	0.95	1.00	1.0
140	5.87	4.55	5.05	3.55	4.85	3.05	1.00	0.91
187	5.71	5.79	4.95	2.55	4.71	1.00	0.95	1.0
228	6.35	2.71	5.25	4.65	4.71	1.5	1.5	1.0
310	6.05	4.87	5.05	5.55	5.35	0.9	1.2	0.91
208*	6.25	4.71	3.85	3.55	4.55	0.9	1.1	1.0
211*	4.55	3.65	3.85	3.55	4.35	0.9	1.0	1.08
217*	4.70	3.60	5.10	5.70	6.50	1.0	1.0	1.0
6†	6.10	4.60	5.30	4.70	5.10	1.0	0.9	1.08

* From human cases. † Virulent strain from bovine mastitis.

lytic circle varies, from 1 to 4 or more millimeters. It may be grayish or slightly turbid. The margin of the hemolytic zone is fairly sharp, tho an occasional exception shows a margin shading off gradually into the surrounding blood. Such zones are as a rule much larger than the ordinary type. According to the description and classification of Smith and Brown this type of hemolysis would correspond to that observed with their Type B.

Tho all these strains belong in the hemolytic group the degree and character of hemolysis in all instances may not be uniform. The strains, on the basis of hemolysis, have arbitrarily been arranged in 4 groups as follows: 1st, wide clear hemolytic circle, from 2 to 4 or more mm. in diameter; 2nd, clear hemolytic circle from 1 to 3 mm. across, with diffuse and indefinite margin; 3rd, small clear hemolytic

circle from 1 to 2 mm. across with sharp margin; 4th, gray-green or hazy hemolytic circle from 1 to 1.5 mm. across, hemolysis often incomplete in circle. The 1st group passes more or less gradually into the 2nd group; the 2nd into the 3rd, and the 3rd into the 4th. This grouping demonstrates that there is a more or less gradual transition from 1 type to the other, and indicates, as was observed, that a strain may change to some extent its type of hemolysis. After carrying these cultures through many generations for a period of 9 months changes from one type to another were not uncommon, as for instance from the 3rd to the 4th; but a clear-cut transition from the clear hemolytic type to a strictly nonhemolytic or a feebly hemolytic organism was not observed. On the whole, they preserve their individual characteristics with considerable, tho not absolute, regularity. In the animal experiments to be detailed transitions from the hemolytic to a strictly nonhemolytic type were likewise not observed, tho slight changes in the type of hemolysis did occur. These observations[9] on the whole are in accord with my previous observations on the hemolytic properties of pathogenic streptococci. They also coincide with the notations of Smith and Brown on this point: "The hemolytic activity of the B types has remained fairly constant. In no case has it disappeared. Strain B-15 from a cow forms an apparent exception, but this strain has in the course of our studies split up into a series of forms differing in their laking capacity, some being nonhemolytic at present."

A study has been made of this group of streptococci as to their ability to resist heat. In connection with our work on the Chicago and Batavia milk epidemics[10] in 1912 I tested the heat resistance of a number of the strains isolated from human sources, and also of one from a suspicious cow with mastitis. These strains all were readily killed at 60 C. after an exposure of 30 minutes. This test was made in order to determine whether or not they could resist the process of pasteurization. Their resistance to lower temperatures was not determined at that time.

Since then the work of Ayer and Johnson[11] on this subject has appeared and is important in this connection. These writers show that a wide variation occurs in the thermal death point of strains of streptococci under conditions similar to pasteurization. For example, 33.07% of their 139 strains of streptococci were able to survive pasteurization temperature (145 F) for 30 minutes; 2.58% were able to withstand 71.1 C. (160 F.), and all were killed only at 73.9 C. (165 F.). They roughly classify the streptococci according to chain-formation into typical and atypical organisms. The latter were decidedly more resistant to heat than the former, only 1 of 22 strains of the typical class resisting a temperature of 62.8 C. (145 F.). The properties of pathogenesis and

[9] Davis: Jour. Infect. Dis., 1913, 12, p. 386.
[10] Davis: Jour. Am. Med. Assn., 1912, 27, p. 1852.
[11] Jour. Agr. Research, 1914, 2, p. 321.

hemolysis were not correlated with the heat resistance in their study. These organisms were isolated from the mouth, feces, and udder of the cow and from milk and cream.

In determining the thermal death point of these streptococci I used practically the same technic as that used by Ayer and Johnson, as follows: Broth cultures were grown in the incubator for from 18 to 24 hours. From each culture 4 drops in a small pipet were inoculated directly into the milk in the milk tubes. Such inoculated milk tubes were placed in a water bath held at a constant required temperature for 30 minutes. The temperature was controlled by a second standard thermometer in a tube of milk in the water bath. The tubes after heating were removed and immediately cooled to 10 C., or lower, in water; then incubated at 37 C. and observed from day to day for several days for growth.

In this manner the thermal death point of 74 strains of the hemolytic streptococci isolated from milk was determined, as summarized in Table 3. In this table the heat resistance of 24 strains of pathogenic hemolytic streptococci which

TABLE 3

TEMPERATURE RESISTANCE OF MILK STREPTOCOCCI AND PATHOGENIC STREPTOCOCCI

Temperature, 30 Minutes	74 Strains of Hemolytic Milk Streptococci		24 Strains of Hemolytic Pathogenic Streptococci	
	Number of Strains Alive	Number of Strains Killed	Number of Strains Alive	Number of Strains Killed
115 F. 46.1 C.	74	0	24	0
120 F. 48.9 C.	74	0	24	0
125 F. 51.7 C.	74	0	10	10
130 F. 54.5 C.	74	0	10	14
135 F. 57.2 C.	74	0	2	22
140 F. 60 C.	74	0	0	24
145 F. 62.8 C.	71	3	0	24
150 F. 65.6 C.	53	21	0	24
155 F. 68.3 C.	20	54	0	24
160 F. 71.1 C.	0	74	0	24
165 F. 73.9 C.	0	74	0	24

I have isolated from various lesions and whose pathogenicity has been determined on rabbits is also presented. These were all of human origin, except 2 strains from diseased udders of cows, one isolated during the Chicago epidemic of sore throat in 1911, the other in 1914 in the epidemic at Jacksonville, Illinois. These organisms were quite like the strains isolated from human lesions of various kinds and they find their place in this group. They were pathogenic for rabbits. Five strains were isolated from human cases, either from throats or from peritoneal fluid at autopsy during the Chicago and Jacksonville epidemics.

From Table 3 it is evident that the pathogenic streptococci are decidedly less resistant to heat than the milk streptococci, the thermal death point of the former ranging from 48.9 to 60 C. (120 to 140 F.) and the latter from 60 to 71.1 C. (140 to 160 F.). This reaction is strikingly definite. The milk streptococci here tested were obtained from pasteurized milk, except the 18 strains isolated from certified milk. One would expect the process of pasteurization to have a selective action in killing off the less resistant strains of hemolytic streptococci. The results in Table 3 show that this does not seem to have been true. In 40% of the certified samples the hemolytic cocci were found; in only 1 other dairy was this percentage surpassed (Dairy G. 45.4%). The average number

of streptococci to the cubic centimeter of milk in those certified samples which contained them occupied an intermediate position in relation to the pasteurized samples. A comparison of the number of hemolytic streptococci from the same milk before and that after pasteurization, tho desirable, could not be undertaken during this investigation.

These results, therefore, should not be interpreted as indicating the heat resistance of all the streptococci that might come directly from the udder of the cow and from the usual sources of milk contamination. The results with the certified milk should afford such data, but the number of such strains (18) is small on which to base a general conclusion.

The thermal death point of the human strains was tested carefully at 2 different times during an interval of 9 months. No important differences were noted between the two series of observations. From the data in the table the necessity of efficient pasteurization is self-evident.

I have gained the impression from many observations that the virulence for rabbits of a given strain of hemolytic streptococci runs rather parallel with its virulence for man. Hemolytic streptococci highly virulent for men are highly virulent for rabbits; when slightly virulent for men they have little virulence for rabbits. In my own work on streptococcal infections in general these animals have proved themselves invaluable and highly superior to guinea-pigs for the determination of virulence. The importance of this test in the study of streptococci from milk and from bovine sources is striking. Rabbits were therefore used regularly in this work and all strains as soon as possible after isolation, were inoculated intravenously.

Streptococci vary markedly in their virulence. The ordinary hemolytic Streptococcus pyogenes is highly virulent and the growth from one 24-hour small blood-agar slant usually makes the animal very ill and often kills in a few days. With many strains ½ tube or even ⅕ or ⅛ of a tube will cause acute arthritis. I have observed some strongly hemolytic streptococci which when inoculated in doses of 2 slant tubes would not kill or produce lesions in a rabbit. Such cocci are exceptional coming from human lesions. It was therefore decided in testing virulence to inject routinely two 24-hour blood-agar slants into young rabbits weighing from 800 to 1,000 gm. Strains which in this dosage did not affect such animals were considered avirulent. This is an arbitrary method of determining virulence, but some standard must be adopted. Smith and Brown in their work used 1 c.c. of a 24-hour broth culture injected into the ear vein of rabbits. This, as they correctly state, is a much smaller dose than is usually employed for such purposes. I have not been able to use successfully plain broth cultures because so many of the pathogenic streptococci grow poorly in this medium. Consequently when not using slant cultures I have used serum broth (1:5), which yields a good growth with all strains.

It is perhaps not quite right to compare broth cultures and slant cultures but estimation that the growth from 1 small blood slant is equivalent to 8 c.c. of a serum-broth culture makes our doses of 2 slants equivalent to 4 c.c. of a serum-broth culture.

Each of 85 strains of the hemolytic cocci were thus injected into the ear vein of a young rabbit of stated weight. The animals were permitted to live 10 days; they were then killed and examined. Cultures were made in all cases from the heart blood, and in most cases from the gallbladder; also from any suspicious lesion, especially joint lesions.

With the dosage as given 15 strains produced lesions visible at autopsy. These involved joints and on the whole were mild. Positive cultures from joints were obtained in all cases save one. Two animals died, one on the 7th, the other on the 9th day after inoculation and streptococci were isolated from the elbow in one and from the wrist in the other. The heart blood was negative in both.

The virulence of each of these 15 strains after the 1st rabbit passage was tested still further. Two cubic centimeters of a 24-hour serum-broth culture of each were injected into the ear vein of rabbits. Eleven animals killed after 10 days showed no lesions. One died in 48 hours and streptococci were grown from the heart blood. The 3 remaining animals, killed after 10 days, revealed in 2 cases pus in the left wrist from which in each case streptococci were grown pure; in the third rabbit, pus was found in the right knee with positive culture. These 4 strains after the two animal passages were again injected in doses of 1 c.c. of a 24-hour serum-broth culture into rabbits. With this dosage no results were obtained.

From these results it is evident that all the strains in this series are relatively avirulent. None could be classed in this respect with the human types of hemolytic streptococci as found in sore-throat epidemics. It is to be noted, however, that when large doses are used there is variation in their pathogenicity, some of the strains approaching but not attaining the standard we have arbitrarily set for an organism's being called virulent. If this be true, might not milk or bovine strains, occasionally at least, attain still higher degrees of virulence for rabbits and in this respect fall in the human group?

A comparison of the 84 strains of hemolytic streptococci from milk and 24 of hemolytic streptococci from human sources was here made, the chief points of which are presented in Table 4. It should be noted that the statements made in the table under the various properties are not in every regard absolute. In the large series of streptococci of milk origin there are some which in one or more points might be properly classed with the human type, and vice versa. Each strain must be studied by itself.

The human virulent streptococci when grown in milk are more spherical, while the milk streptococci tend to be more elongated, but

since many exceptions appear, this characteristic has little value for determination purposes. This is true also of the arrangement of the streptococci in milk. The human strains commonly form long chains, while the milk strains appear in diplococci and short chains. Changing the media will often alter this characteristic markedly.

Capsules are present at times in the virulent human strain, as was particularly true of some of the epidemic strains. They may be lost under artificial conditions and acquired again by animal passage. The capsule appears to be strikingly persistent in some strains. None of the milk strains possessed a capsule. In this series the encapsulated streptococi were always highly virulent for rabbits. However, hemolytic streptococci of the human type without capsules may also be highly virulent for rabbits.

TABLE 4

COMPARISON OF HEMOLYTIC HUMAN AND MILK STREPTOCOCCI

Characteristics Studied	Human Type	Milk Strains
Shape	Spherical or slightly elongated	More elongated
Arrangement	Marked chain-formation	Diplococci or short chains
Capsules	May or may not be present	Not present
Growth in plain broth	Often not very abundant	More abundant
Groth at 20 C. in milk	Very slow or not at all	Good growth as a rule
Growth at 37 C. in milk	More slowly acidified, with, or often without, coagulation	Rapidly acidified and coagulated
Acidity in sugar broths	Moderate	Higher
Hemolysis	Zone wide and usually clear	Zone narrower and at times turbid (many exceptions)
Heat resistance, 30 minutes	120-140 F. (48.9-60 C.)	140-160 F. (60-71.1 C.)
Pathogenicity for rabbits	Marked	Very little or none
Pathogenicity for cow	Marked	Very little

In plain broth, growth of many human strains is not abundant, tho there are notable exceptions. The milk strains grow more vigorously and profusely in this medium. At 20 C. in milk the growth of the human strains is slight or at a stand-still, while the milk strains grow well. At 37 C. in milk the human strains acidify with or without coagulation; the milk strains acidify more rapidly and coagulate in every instance, the medium usually being pink at the top and white beneath.

In the fermentable carbohydrate broths the milk strains grow more profusely, acidify more quickly, with generally a higher terminal acidity.

Comparing the 2 groups with reference to hemolysing property we find no really important or constant differences. Those from human sources possess a somewhat wider and clearer zone than those from the milk, but there are so many exceptions that such a statement has little value. Just as there are slight differences, already noted, between milk strains in the character of the hemolytic zone, so there exist similar differences between many of the human strains. Such have been referred to in my earlier papers,[12] when I was studying the properties of the epidemic streptococci. Between hemolytic area and

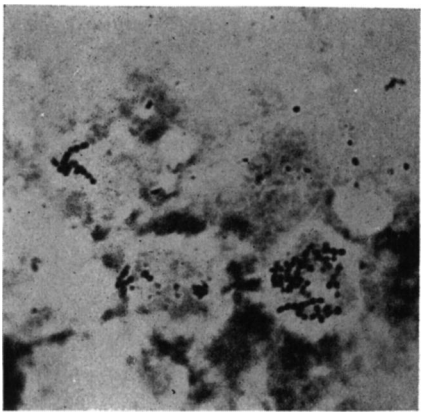

Figure 1

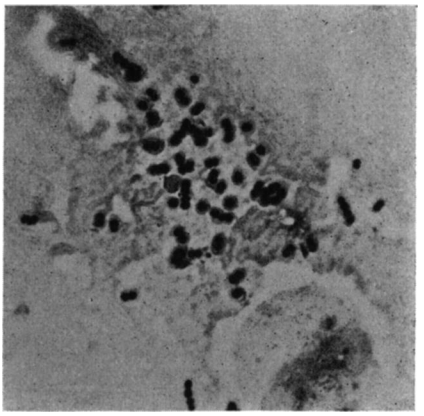

Figure 2

Fig. 1. Hemolytic streptococci stained directly in milk from cow suffering with mastitis (5th week). Phagocytosis is evident. Capsules very doubtful.

Fig. 2. Same strain as shown in Fig. 1. In the body of the rabbit for which it was virulent it acquired the distinct capsules shown.

pathogenicity there exists no consistent parallelism. Indeed, it may happen that as the virulence is raised the zone of hemolysis becomes narrower. This may be due to the production of a less diffusible hemolysis, which need not necessarily be less toxic. But there is no good reason to think that the symptoms of streptococcal infections are to any appreciable degree dependent on the production of a streptolysin.

As regards heat resistance the two series have already been compared. It may be stated again that the human pathogens appear to be decidedly more sensitive to heat.

[12] Jour. Am. Med. Assn., 1912, 58, p. 1852.

I would repeat also that pathogenicity for rabbits seems to be a most valuable character for determining the sanitary significance of a streptococcus from human or bovine sources. There is no evidence at present to lead one to believe that a hemolytic streptococcus that is avirulent for rabbits has any pathogenic properties for man. On the other hand, all the dangerous streptococci isolated from the epidemics of sore throat are highly virulent for rabbits. The importance of the virulence for rabbits, therefore, in connection with studies of these milk epidemics cannot be over emphasized.

One of these hemolytic milk strains was injected by Dr. Mathers[13] into the udder of a cow, 2 c.c. of a 24-hour milk culture being used. A mild inflammation resulted; the streptococci increased in number for 2 days, then rapidly decreased and after 15 days the milk was again sterile. The number of leukocytes in general paralleled the number of streptococci; on the second day after injection they were most numerous, numbering at that time 6,200,000 to the cubic centimeter, and on the 10th day they had reached normal (43,000). A careful comparison of the behaviors of the different varieties of streptococci when injected into the udder was made by Mathers. It suffices here to state that hemolytic streptococci of the human type, coming directly either from human lesions or from a diseased udder, are decidedly more virulent for the cow than are the hemolytic milk strains.

This comparison leads us to ask whether or not we are justified in making a clear-cut distinction between the bovine type and the human type of hemolytic streptococci. Brown and Smith noted that their bovine strains had certain characteristics which distinguished them from the strains coming from human sources, with the exception of 2 strains from cows which conformed with the human type. The chief points that they make in their distinction are as follows: The bovine strains are not pathogenic for rabbits; they produce a higher degree of acidity than the human strain; in milk they produce a firm coagulum in 3 days, whereas in the case of the human strains the milk remains fluid or only a slight coagulum forms at the bottom of the tube. In these respects our milk strains agree very well with Smith and Brown's bovine strains, and probably belong to the same general group. The 85 milk strains tested may be assumed to be of bovine origin, and in contrast to the pathogenic human types, may be referred to as the bovine type of hemolytic streptococci. Most of them probably originated from the feces, skin, hair, mouth, etc., of the cow, some possibly directly from the udder.

[13] Jour. Infect. Dis., 1916, 19, p. 222.

The relationship of these hemolytic milk strains to the Streptococcus-lacticus type is apparently very close. The latter are commonly nonhemolytic or very feebly hemolytic, and often produce a green colony and a green zone on blood plates. Many strains, however, form small gray colonies without any appreciable alteration of the blood. Exclusive of hemolysis the two types are practically identical. They are both avirulent for rabbits and, as stated previously, Mathers has shown that when injected into the udder of a cow they both produce a similar mild type of mastitis, which clears up in the course of from 1 to 2 weeks.

Of the 85 strains isolated from the milk none had properties which would justify one in considering it of human origin. It is quite probable, however, that if a great number of hemolytic strains were isolated from mixed unpasteurized market milk the human type, which could be definitely identified, would be found. Such a strain might originate directly from man or from a cow suffering with mastitis caused by streptococci of the human type.

The question naturally arises as to whether or not all epidemics of sore throat are caused by streptococci from human sources that gain entrance into the udder and grow there. That this is possible is clear from our earlier experimental work and that it may actually occur is supported by the work of Smith and Brown and others. The data at hand are not, however, sufficient to exclude the possibility of bovine streptococci attaining virulence for man. Man may be susceptible to both bovine and human streptococci just as he is to both bovine and human tubercle bacilli. This possibility should not be lost sight of. The data presented in this paper indicate different degrees of virulence for rabbits on the part of the milk streptococci.

Milk containing virulent hemolytic streptococci of the human type need not necessarily be dangerous to use, tho of course such milk should be condemned. Clinical experience is against the view that in man streptococci from skin and many other lesions are always or even commonly concerned in causing throat infections. It may happen that the udder becomes infected, for example, from a streptococcal infection of the finger of a milker. Such streptococci, tho virulent, might not be able to cause an epidemic of sore throat. There is here involved the question of specific-tissue affinity, a problem that Rosenow[14] has recently discussed.

[14] Jour. Am. Med. Assn., 1915, 65, p. 1687.

The question of variability and mutation and its bearing on the data here presented is a large and difficult subject, and the experiments and observations in this paper were not designed to bear directly on this problem. It may be said that during the time and under the various conditions that the different strains of streptococci were observed, only slight variations in certain properties, as for example changes in sugar reactions, were occasionally noted. No radical change was observed in the character of hemolysis on human-blood-agar plates in any of the strains. I have, however, observed slight loss or gain in hemolytic power in some of the strains, in others slight alterations in the character of hemolysis, as has already been pointed out. That virulence of streptococci can be altered by repeated transfer from animal to animal, or by growth on artificial media, is a well known phenomenon. The variations observed in the study of these streptococci were those commonly seen by bacteriologists in their daily work in the laboratory in connection with different varieties of bacteria. Any alterations suggesting the origin of mutants were not observed. I have not obtained the impression from this or previous study on streptococci that fundamental variations or mutations play a practical rôle in problems of the kind here considered.

In regard to the variation of minor cultural details of strains, it was noted in studying streptococci during the Chicago epidemic, that in body fluids particularly the organisms tended to develop different properties from those of many cocci from the throat of the same individual. I quote[15] as follows:

"On sugars, milk and other mediums these two varieties grow practically alike. The relation of these two types is undoubtedly a very close one and certain facts would seem to indicate that the one is simply a modified form of the other. When grown on ordinary mediums the encapsulated coccus—at least some strains—loses its capsule and the growth on slants is less profuse and moist. By animal passage the capsule and other properties mentioned return. The profuse moist appearance of the colonies, it may be stated, seems to be dependent on the capsular substance. While this seems to be true of some strains other strains maintain their peculiar characteristics for a long time (several months) with little or no change."

In this connection Smith and Brown[3] say regarding their streptococci that "spontaneous changes in cultural characters do not proceed rapidly enough if they go on at all to interfere with current bacteriologic methods."

[15] Davis: Jour. Am. Med. Assn., 1912, 58, p. 1852.

SUMMARY

Hemolytic streptococci having a wide clear zone occur commonly in both pasteurized and unpasteurized (certified) milk. These strains vary among themselves. They are more resistant to heat than human strains of hemolytic streptococci, and possess little or no virulence for rabbits, therefore in all probability none for man. They rapidly acidify and coagulate milk and grow well at 20 C. They form short or long chains, but as seen in milk often appear in pairs or a chain of few elements. While they are definitely hemolytic (Type B. Smith and Brown), the characteristics of the hemolytic zone on plates may vary in certain respects.

The milk strains are different from certain strains of hemolytic streptococci found at times in diseased udders in cows. These latter resemble the strains of hemolytic streptococci from human sources, and are virulent for rabbits.

There is no reason to consider that these organisms have any sanitary significance. The importance, however, of certain types of hemolytic streptococci in relation to epidemics of sore throat makes it necessary to study carefully all such organisms in milk.

By itself the hemolytic property has no more value for identification purposes than many other characteristics, but is greatly important on account of the practical value of the blood-agar-plate method as a means of initial separation of human type strains from the many strains of nonhemolytic and feebly hemolytic streptococci found in milk.